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Patent

5/13/97

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:	)	Group Art Unit: 1804
Gjerset and Sobol	)	Examiner: Low, C.
Serial No.: 08/335,461	)	
Filed: November 7, 1994	)	
For: ENHANCING THE SENSITIVITY OF TUMOR CELLS TO THERAPIES	)	

APPELLANT'S BRIEF ON APPEAL
SUBMITTED PURSUANT TO 37 C.F.R. § 1.192

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicant hereby appeals the Examiner's final rejection mailed June 24, 1996. Enclosed please find a Request for Oral

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CERTIFICATE OF MAILING

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as Express Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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CASES

STATUTES

MISCELLANEOUS

STATUS OF THE CLAIMS

Claims 1, 2, 4-20 and 23 are pending (*see* Appendix).

All pending claims are rejected under 35 U.S.C. § 112, first paragraph, as the specification allegedly does not contain a written description of the claimed invention.

All pending claims are rejected under 35 U.S.C. § 112, second paragraph, as allegedly failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

All pending claims are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Cheng et al., Srivastava, Moossa et al., Wu et al., Malkin et al., Chen et al., Nabel et al., Itoh et al. and Eppstein et al.

STATUS OF AMENDMENTS

Claims 3, 21 and 22 have been canceled without prejudice to further prosecution thereof. Amendments to claims 1, 2, 6, 9, 10, 12-15, 17-20 and 23 were filed after the Final Office Action. These amendments were made to narrow issues for appeal and to adopt the Examiner's suggestions in the Final

Office Action. Applicant submits that the amendments do not necessitate a new search or raise any issue of new matter. The Examiner has indicated in an Advisory Action mailed March 11, 1997 that the proposed amendments will be entered upon the filing of an Appeal Brief.

SUMMARY OF THE INVENTION

The present invention relates to a process of enhancing the therapeutic effect of a cancer therapy such as chemotherapy or radiation therapy.

It has been known in the art that many types of human cancer cells carry mutations in the p53 gene. Some prior art references have shown that the introduction of a wild-type p53 gene into a variety of cancer cells reduces their growth rate. p53 gene is therefore known as a tumor suppressor gene.

Within the scope of this invention, Applicant has discovered a new function of the wild type p53 gene, namely, its ability to increase the sensitivity of a tumor cell lacking an endogenous wild-type p53 gene to cancer therapies. Therefore, the introduction of a wild-type p53 gene or protein into a tumor

cell deficient in its wild-type p53 gene may be combined with a conventional cancer therapy to increase the therapeutic effect of the conventional cancer therapy, i.e., to kill cancer cells and/or to suppress tumor growth.

In that regard, this invention features a process of increasing the therapeutic effect of a cancer therapy by introducing a wild-type p53 gene into a tumor cell lacking an endogenous wild-type p53 gene and expressing the heterologous wild-type p53 gene before subjecting the tumor cell to the cancer therapy.

Cancer therapies which can be augmented by this process include radiation therapy, chemotherapy, immunotherapy, cryotherapy and hyperthermia. Tumors which can be treated by this process include leukemia, lymphoma, ovarian carcinoma, osteogenic sarcoma, lung carcinoma, colorectal carcinoma, hepatocellular carcinoma, glioblastoma, prostate cancer, breast cancer, bladder cancer, kidney cancer, pancreatic cancer, gastric cancer, esophageal cancer, anal cancer, biliary cancer, and urogenital cancer.

In a preferred embodiment, the wild-type p53 gene is included in a vector such as adenovirus vector, retroviral vector, adeno-associated virus vector, herpes virus vector, vaccinia virus vector and papilloma virus vector.

In other preferred embodiments, the wild-type p53 gene is coupled to a virus capsid or particle, encapsulated in a liposome, or conjugated with a ligand such as an asialoglycoprotein. The wild-type p53 gene may be introduced to the tumor cell with an aerosolized preparation or by direct injection, intra-arterial infusion, intravenous infusion, or intracavitary infusion.

Alternatively, a wild-type p53 protein is introduced into the tumor cell in place of the wild-type p53 gene.

ISSUES

(a) Whether pending claims 1, 2, 4-20 and 23 are supported by a written description in the original specification whereas these claims are of similar scope and wordings with the original claims?

(b) Whether claims 1, 2, 4-20 and 23 clearly and precisely define the invention whereas terms and phrases well known to those skilled in the art are used?

(c) Whether the invention of claims 1, 2, 4-20 and 23 is obvious in view of the nine references cited by the Examiner whereas these references only motivate one skilled in the art to use a wild-type p53 gene to suppress tumor growth but teach away from using the gene to sensitize tumor cells to cancer therapies?

GROUPING OF CLAIMS

Pending claims 1, 2, 4-20 and 23 will be considered in one group.

ARGUMENT

I. THE SPECIFICATION CLEARLY DESCRIBES THE INVENTION OF CLAIMS 1, 2, 4-20 AND 23

In the Final Office Action mailed June 24, 1996, the Examiner acknowledged the utility of the claimed invention but rejected the pending claims for inadequate written description,

stating on page 11 of the Final Office Action that "[t]he rejection is not one for lack of utility but for inadequate written description of the invention."

The Examiner also stated on page 2 of the Final Office Action:

> Claims 1-23 are rejected under 35 U.S.C. 112, first paragraph, as the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time the application was filed.

Applicant respectfully traverses this rejection.

A. Claims 1, 2, 4-20 and 23 define an invention that is clearly conveyed to those skilled in the art at the time the application was filed

The invention claimed in claims 1, 4-20 and 23 features a process of increasing the therapeutic effect of a cancer therapy by introducing a wild-type p53 gene into a tumor cell lacking an endogenous wild-type p53 gene. This process expresses the heterologous wild-type p53 gene in the tumor cell before subjecting the tumor cell to the cancer therapy. Claim 2 substitutes the wild-type p53 gene with a wild-type p53 protein.

"A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by the disclosure of an application as filed." MPEP § 2163.01. "Whenever the issue arises, the fundamental factual inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed." MPEP § 2163.02.

Applying the factual inquiry to this case, one skilled in the art would find that claims 1, 2, 4-20 and 23 are described in both the original claims and the original specification of this application.

For example, the original claims 1 and 22 state:

> (1) Method of increasing the effect of a cancer therapy, comprising the steps of:
>
> delivering wild-type therapy-sensitizing gene activity to a tumor cell characterized by loss of said wild-type therapy-sensitizing gene activity, and
>
> subjecting said tumor cell to said cancer therapy.
>
> (22) The method of claim 1, wherein said therapy-sensitizing gene activity is p53 therapy-sensitizing activity.

Original claims constitute their own description, and later added claims of similar scope and wording are described

thereby. In re Koller, 204 U.S.P.Q. 702, 706 (CCPA 1980).

Pending claims 1 and 2 are of similar scope and wording to the original claim 22. The only difference between pending claims 1 and 2 and the original claim 22 is that the claim scope has been narrowed from the "p53 therapy-sensitizing activity" to the "wild-type p53 gene" in claim 1 and the "wild-type p53 protein" in claim 2. In that regard, the original specification explains that the p53 therapy-sensitizing activity is embodied in the wild-type p53 gene and the wild-type p53 protein.

The original specification on pages 13-16 illustrates a general procedure for carrying out the method for enhancing the effect of a cancer therapy with a wild-type p53 gene or protein. In addition, Example 1 of the specification describes introducing a wild-type p53 gene into a tumor cell. Example 2 of the specification describes introducing a wild-type p53 protein into a tumor cell.

In Example 4, a plasmid containing a wild-type p53 gene was transfected into glioblastoma cells which do not contain endogenous wild-type p53 gene. The wild-type p53 gene was expressed in these cells before these cells were treated with a

chemotherapy drug, cisplatin. It was described in the specification that the tumor cells with the transfected wild-type p53 gene were considerably more sensitive to the chemotherapy drug than those not having the wild-type p53 gene.

In Example 5, the cells described in Example 4 were subject to gamma radiation. It was described in the specification that the tumor cells with the transfected wild-type p53 gene were considerably more sensitive to the radiation therapy than those not having the wild-type p53 gene.

In addition, pending claims 4-20 are of similar scope and wording to the original claims 4-20. Pending claim 23 is of similar scope and wording to the original claim 17.

Accordingly, Applicant submits that one skilled in the art reading the original claims and the original disclosure would recognize that the inventors had possession at the filing of this application the invention covered by the pending claims 1, 2, 4-20 and 23.

B. The Examiner has failed to carry the burden of proof

> The examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 191 U.S.P.Q. 90, 98 (CCPA 1976); Ex parte Sorensen, 3 U.S.P.Q.2d 1462, 1463 (Bd. Pat. App. & Inter. 1987).
>
> * * *
>
> Any time an examiner bases a rejection of a claim . . . on the lack of a written description, the examiner should:
>
> (1) identify the claim limitation not described; and
>
> (2) provide reasons why persons skilled in the art at the time the application was filed would not have recognized the description of this limitation in the disclosure of the application as filed.

MPEP § 2163.04.

In the Final Office Action, however, the Examiner did not identify any claim limitation in any of claims 1-23 which is not described in the original specification or the original claims. Neither did the Examiner explain why those skilled in the art would not have recognized any claim limitation in any of claims 1-23 in the original specification or the original claims.

For the above stated reasons, Applicant submits that pending claims 1, 2, 4-20 and 23 are clearly and fully described in the specification.

II. CLAIMS 1, 2 AND 9 CLEARLY AND PRECISELY DEFINE THE INVENTION TO THOSE SKILLED IN THE ART

In an Advisory Action mailed March 11, 1997, the Examiner repeated his rejection against claims 1, 2 and 9 under 35 U.S.C. § 112, second paragraph.

According to the Examiner, claim 1 is indefinite because the claim does not give definite answers to the following five questions:

(1) What is the therapeutic effect of a cancer therapy?

(2) What effect does the wild-type p53 gene accomplish?

(3) Whether lethal doses of the cancer therapy is included or excluded?

(4) What is the wild-type p53 gene?

(5) How is the wild-type p53 gene delivered?

The Examiner also stated that claim 2 is indefinite because the claim does not indicate what DNA is or is not the "wild-type p53 gene." In addition, the Examiner stated that claim 9 is indefinite because it is not clear how the different types of cancer cells listed in claim 9 differ from each other.

Applicant respectfully traverses this rejection.

A. The terms and phrases used in claims 1, 2 and 9 have well defined meanings to those skilled in the art

According to MPEP § 2173.02:

> Definiteness of claim language must be analyzed, not in a vacuum, but in light of (1) the content of the particular application disclosure, (2) the teachings of the prior art, and (3) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

In that regard, claim 1 clearly answers the five questions raised by the Examiner when read in light of the specification and the teachings of the prior art.

(1) It is well known to those skilled in the art that the therapeutic effect of a cancer therapy is to kill cancer cells and/or repress tumor growth. A patent need not teach, and preferably omits, what is well known in the art. Hybritech Inc. v. Monoclonal Antibodies, Inc., 231 U.S.P.Q. 81, 94 (Fed. Cir. 1986).

(2) Claim 1 and the specification made it clear that the effect accomplished by the wild-type p53 gene is to sensitize a cancer cell lacking an endogenous wild-type p53 gene to conventional cancer therapy. How does the wild-type p53 gene accomplish the effect is irrelevant here because "it is axiomatic

that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests." Fromson v. Advance Offset Plate, Inc., 219 U.S.P.Q. 1137, 1140 (Fed. Cir. 1983).

(3) As described in the specification, the determination of effective dosage levels of the cancer therapy and the wild-type p53 gene is within the ambit of one skilled in the art. The dosage will vary depending on elements such as the condition of the patient and the cancer being treated. According to MPEP § 2173.04:

> Breadth of a claim is not to be equated with indefiniteness. In re Miller, 169 U.S.P.Q. 597 (CCPA 1971). If the scope of the subject matter embraced by the claims is clear, and if applicants have not other wise indicated that they intend the invention to be of a scope different from that defined in the claims, then the claims comply with 35 U.S.C. § 112, second paragraph.

Because one skilled in the art would know how to adjust the dosage to treat a particular cancer in a particular patient, claim 1 does not become indefinite because a dosage is not specified there.

(4) About half of the cancers have been identified to contain mutated p53 genes since the discovery of p53 gene in the

1980s. As shown by the references cited by the Examiner in the section 103 rejection, those skilled in the art are familiar with what is a wild-type p53 gene and what is a mutated p53 gene. "An inventor need not, however, explain every detail since he is speaking to those skilled in the art. What is conventional knowledge will be read into the disclosure." In re Howarth, 210 U.S.P.Q. 689, 691 (CCPA 1981).

(5) The principle spelled out in In re Howarth applies to the introduction of the wild-type p53 gene into a tumor cell as well. As described in Example 1 of the specification, numerous techniques for delivering a gene into a cell are known to those skilled in the art. It is unnecessary for purposes of 35 U.S.C. § 112, second paragraph, to state in claim 1 a specific method of deliverance because "[n]ot every last detail is to be described, else patent specifications would turn into production specifications, which they were never intended to be." In re Gay, 135 U.S.P.Q. 311, 316 (CCPA 1962).

The Examiner objected to the inclusion of ovarian cancer cells and urogenital cancer cells in the same Markush group. The Examiner also objected to the inclusion of colorectal

cancer cells and anal cancer cells in the same Markush group. However, the inclusion of these elements in the same Markush group is permitted by MPEP § 2173.05(h):

> The use of Markush claims of diminishing scope should not, in itself, be considered a sufficient basis for objection to or rejection of claims. ... Similarly, the double inclusion of an element by members of a Markush group is not, in itself, sufficient basis for objection to or rejection of claims. ... The mere fact that a compound may be embraced by more than one member of a Markush group recited in the claim does not necessarily render the scope of the claim unclear. For example, the Markush group, "selected from the group consisting of amino, halogen, nitro, chloro and alkyl" should be acceptable even though "halogen" is generic to "chloro."
>
> * * *
>
> it is sufficient if the members of the group are disclosed in the specification to possess at least one property in common which is mainly responsible for their function in the claimed relationship . . .

In this case, the above mentioned cancer cells are included in the same Markush group because they share a common characteristic, i.e., deficiency in their wild-type p53 gene. Therefore, their inclusion in the same Markush group is appropriate even though they overlap with each other.

III. CLAIMS 1, 2, 4-20 AND 23 ARE NOT OBVIOUS IN VIEW OF THE REFERENCES CITED BY THE EXAMINER

The Examiner rejected claims 1-11, 17-20 and 22 under 35 U.S.C. § 103 as allegedly being unpatentable over Cheng et al. taken with Srivastava and Moossa et al. Claims 12-18 and 20 are rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Cheng et al. taken with Srivastava, Moossa et al. and further in view of Wu et al., Malkin et al. and Chen et al. Claims 1-15, 17-20 and 22 are rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Nabel et al. taken with Wu et al., Malkin et al. and Moossa et al. Claim 21 is rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Cheng et al. taken with Srivastava, Moossa et al., or over Nabel et al. taken with Wu et al., Malkin et al. and Moossa et al., and further in view of Itoh et al. Claim 23 is rejected under 35 U.S.C. § 103 as allegedly being unpatentable over Cheng et al. taken with Srivastava, Moossa et al., or over Nabel et al. taken with Wu et al., Malkin et al. and Moossa et al., and further in view of Eppstein et al.

However, given the differences between the present invention and the references cited by the Examiner, the

references fail to support a *prima facie* case of obviousness.

A. The References Cited By The Examiner

Cheng et al. expressed a wild-type p53 gene in the human T leukemia cell line Be-13. The expression of the wild-type p53 gene reduced the growth rate of the leukemia cells, suppressed colony formation of the leukemia cells in methylcellulose cultures, and abrogated the leukemia cells' tumorigenic phenotype in nude mice.

Srivastava described hybrid parvovirus vectors for delivering genes into a cell.

Moossa et al. described conventional cancer therapies such as radiation therapy, chemotherapy, biological therapy, cryotherapy and hyperthermia.

Wu et al. described a targetable gene delivery system for introducing foreign genes into mammalian cells through receptor-mediated endocytosis.

Malkin et al. described that mutations of the *p53* gene occur not only as somatic mutations in human cancers, but also as germ line mutations in some cancer-prone families.

Chen et al. expressed a wild-type p53 gene in a peripheral neuroepithelioma cell line (A673). The expression of the wild-type p53 gene reduced the tumor cells' ability to form colonies in soft agar and tumors in nude mice.

Nabel et al. described delivering proteins to discrete blood vessel segments by catheterization using genetically modified or normal cells or other vector systems.

Itoh et al. isolated cDNA encoding human Fas antigen determinant from human T cell lymphoma KT-3 cells. When they expressed the cDNA in murine T cell lymphoma WR19L or fibroblast L929, the transformed cells were killed by mouse anti-Fas antibody by apoptosis.

Eppstein et al. described liposome formulations which can be used to transport DNA, RNA or polypeptide into cells.

B. The references cited by the Examiner fail to support a *prima facie* case of obviousness when all limitations of the claims are considered

When evaluating a claim for determining obviousness, all limitations of the claim must be evaluated. In re Gulack, 217 U.S.P.Q. 401 (Fed. Cir. 1983).

The claimed invention increases the therapeutic effect of a conventional cancer therapy with a process that contains at least two steps:

(1) deliver a wild-type p53 gene to a tumor cell that has lost its wild-type p53 gene and effect the expression of the wild-type p53 gene; and then

(2) subject the tumor cell to a conventional cancer therapy such as radiation therapy, chemotherapy, biological therapy, cryotherapy or hyperthermia.

The Examiner argued that because Cheng et al. disclosed that the expression of a wild-type p53 gene suppressed the growth of tumor cells *in vivo*; Srivastava disclosed vectors for gene therapy; and Moossa et al. described radiation therapy, chemotherapy and other treatment methods; it would have been obvious to anyone of ordinary skill in the art to deliver a wild-type p53 gene to a cancer patient along with routine cancer therapies. However, none of these references described or suggested combining the two steps of this invention to increase the therapeutic effect of a cancer therapy.

Where one element of the claimed invention is found in

one reference, and another element of the claimed invention is found in another reference, the teachings of the two references can be combined **only if** there is some suggestion or incentive to do so. In re Fine, 5 U.S.P.Q.2d 1596, 1599 (Fed. Cir. 1988). In addition, the motivation or suggestion for combining the teaching must be other than the knowledge learned from the disclosure of the applicant. In re Laskowski, 10 U.S.P.Q.2d 1397, 1398 (Fed. Cir. 1989). In this case, no suggestion or motivation existed in the prior art or was given in the references cited by the Examiner.

Although Chen et al. described that a wild-type p53 gene reduced tumor cells' ability to form colonies in soft agar and tumors in nude mice, they did not describe or suggest using a wild-type p53 gene to make the tumor cells more sensitive to chemotherapy or radiation. Wu et al., Eppstein et al., and Nabel et al. described methods for delivering genes or proteins to cells but did not describe delivering a wild-type p53 gene into tumor cells to sensitize them to cancer therapies. Itoh et al. described cloning the Fas antigen and an antibody against the Fas antigen but did not describe delivering a wild-type Fas gene into

tumor cells to sensitize them to cancer therapies.

The references cited in the Final Office Action each described a part of the claimed process but they did not describe the whole process or suggest combining the parts to make the whole process. The only way the disclosures of Cheng et al. and other references cited by the Examiner can be read to result in the above statement is with benefit of Applicant's disclosure.

Such use of Applicant's disclosure would be improper in an obviousness analysis. Selective hindsight cannot be used to evaluate obviousness. There must be a reason or suggestion in the prior art for selecting the procedure used, other than the knowledge learned from the applicant's disclosure. In re Dow Chem. Co., 5 U.S.P.Q.2d 1529, 1532 (Fed. Cir. 1988).

C. The motive of the prior art references is different from that of the claimed invention

The Examiner argued in the obviousness rejection that Cheng et al. had suppressed the growth of leukemia cells (T-ALL) by introducing a wild-type p53 gene into the tumor cells. Therefore, the motive of Cheng et al. (and Chen et al.) is to use the wild-type p53 gene to reduce the growth rate of tumor cells.

However, the tumor suppression effect of a wild-type p53 gene is different from the therapy sensitization effect of a wild-type p53 gene. Neither Cheng et al. or Chen et al. suggested using the wild-type p53 gene to sensitize tumor cells to cancer therapies.

That the wild-type p53 gene is a tumor suppressor, as reported in Cheng et al. and Chen et al., would not lead one skilled in the art to conclude that the wild-type p53 gene would increase the therapeutic effect of a cancer therapy. On the contrary, the growth suppressive effect of the wild-type p53 gene documented in Cheng et al. and Chen et al. would lead one skilled in the art to expect it to render tumor cells more resistant to conventional chemotherapy and radiotherapy because chemotherapy and radiotherapy preferentially kill or suppress fast growing cells. In that regard, Cheng et al. and Chen et al. teach away from the invention claimed in this application.

Conventional chemotherapeutic and radiation regimens employed for the treatment of cancer are known to work by killing or suppressing cells undergoing rapid growth. Chemotherapeutic drugs and radiation are known to interfere with the mitotic or

cell cycle process required for cell growth and division. It is known in the prior art that nondividing cells and slow-growing cells tend to be less susceptible to chemotherapy and radiation than fast-growing cancer cells. By this rationale, the expression of wild-type p53 gene in tumor cells would be expected to reduce, rather than increase, the sensitivity of the tumor cells to chemotherapy and radiation because wild-type p53 gene suppresses tumor cell growth.

It is improper to combine references where the references teach away from their combination. In re Grasselli, 218 U.S.P.Q. 769, 779 (Fed. Cir. 1983).

D. The claimed invention provides an unexpected result

Unexpected or surprising results achieved by the claimed invention may be strong support for nonobviousness. Lindemann Maschinenfabrik v. American Hoist & Derrick Co., 221 U.S.P.Q. 481, 488 (Fed. Cir. 1984). In this invention, the unexpected result of using a wild-type p53 gene to increase the therapeutic effect of chemotherapy and radiation therapy on tumor cells is shown in Figures 1 and 2, and Examples 4 and 5 of the

specification.

Figure 1 and Example 4 show that tumor cells transduced with a wild-type p53 gene are more sensitive to cisplatin treatment than tumor cells lacking any wild-type p53 gene. Figure 2 and Example 5 show that tumor cells transduced with a wild-type p53 gene are more sensitive to radiation therapy than tumor cells lacking any wild-type p53 gene.

A prior art reference, Vogelstein et al., Cell 70: 523-526, (1992) (a copy is enclosed herein), stated on page 526 that "**p53 mutations** may therefore constitute one of the few oncogenic alterations that **increase** rather than decrease the sensitivity of cells to antitumor agents."

Contrary to the conventional wisdom and the teachings of prior art such as Vogelstein et al., Applicant found that the wild-type p53 gene increases the sensitivity of tumor cells to cancer therapies. This surprising discovery and its application as embodied in claims 1, 2, 4-10 and 23 were unexpected and not obvious from the prior art.

CONCLUSION

In view of the above discussion, Applicant submits that claims 1, 2, 4-20 and 23 are allowable. Applicant respectfully requests that they be allowed and passed to issue.

Respectfully submitted,

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APPENDIX

Claims

1. A method of increasing the therapeutic effect of a cancer therapy, comprising the steps of:

delivering a wild-type p53 gene to a tumor cell which is deficient in its wild-type p53 gene, effecting the expression of said wild-type p53 gene in said tumor cell, and

subjecting said tumor cell to said cancer therapy.

2. A method of increasing the therapeutic effect of a cancer therapy, comprising the steps of:

delivering a wild-type p53 protein to a tumor cell which is deficient in its wild-type p53 gene, and

subjecting said tumor cell to said cancer therapy.

4. The method of claim 1 wherein said cancer therapy is radiation therapy.

5. The method of claim 1 wherein said cancer therapy is chemotherapy.

6. The method of claim 1, wherein said cancer therapy is immunotherapy.

7. The method of claim 1, wherein said cancer therapy is cryotherapy.

8. The method of claim 1, wherein said cancer therapy is hyperthermia.

9. The method of claim 1 wherein said tumor cell is selected from the group consisting of leukemia cell, lymphoma tumor cell, ovarian carcinoma cell, osteogenic sarcoma cell, lung carcinoma cell, colorectal carcinoma cell, hepatocellular carcinoma cell, glioblastoma cell, prostate cancer cell, breast cancer cell, bladder cancer cell, kidney cancer cell, pancreatic cancer cell, gastric cancer cell, esophageal cancer cell, anal cancer cell, biliary cancer cell, and urogenital cancer cell.

10. The method of claim 1, wherein said wild-type p53 gene is in a vector.

11. The method of claim 10, wherein said vector is selected from the group consisting of adenovirus vector, retroviral vector, adeno-associated virus vector, herpes virus vector, vaccinia virus vector and papilloma virus vector.

12. The method of claim 1, wherein said wild-type p53 gene is coupled to a virus capsid or particle.

13. The method of claim 12, wherein said wild-type p53 gene is coupled to said capsid or particle through a polylysine bridge.

14. The method of claim 1, wherein said wild-type p53 gene is encapsulated in a liposome.

15. The method of claim 1, wherein said wild-type p53 gene is conjugated to a ligand.

16. The method of claim 15, wherein said ligand is an asialoglycoprotein.

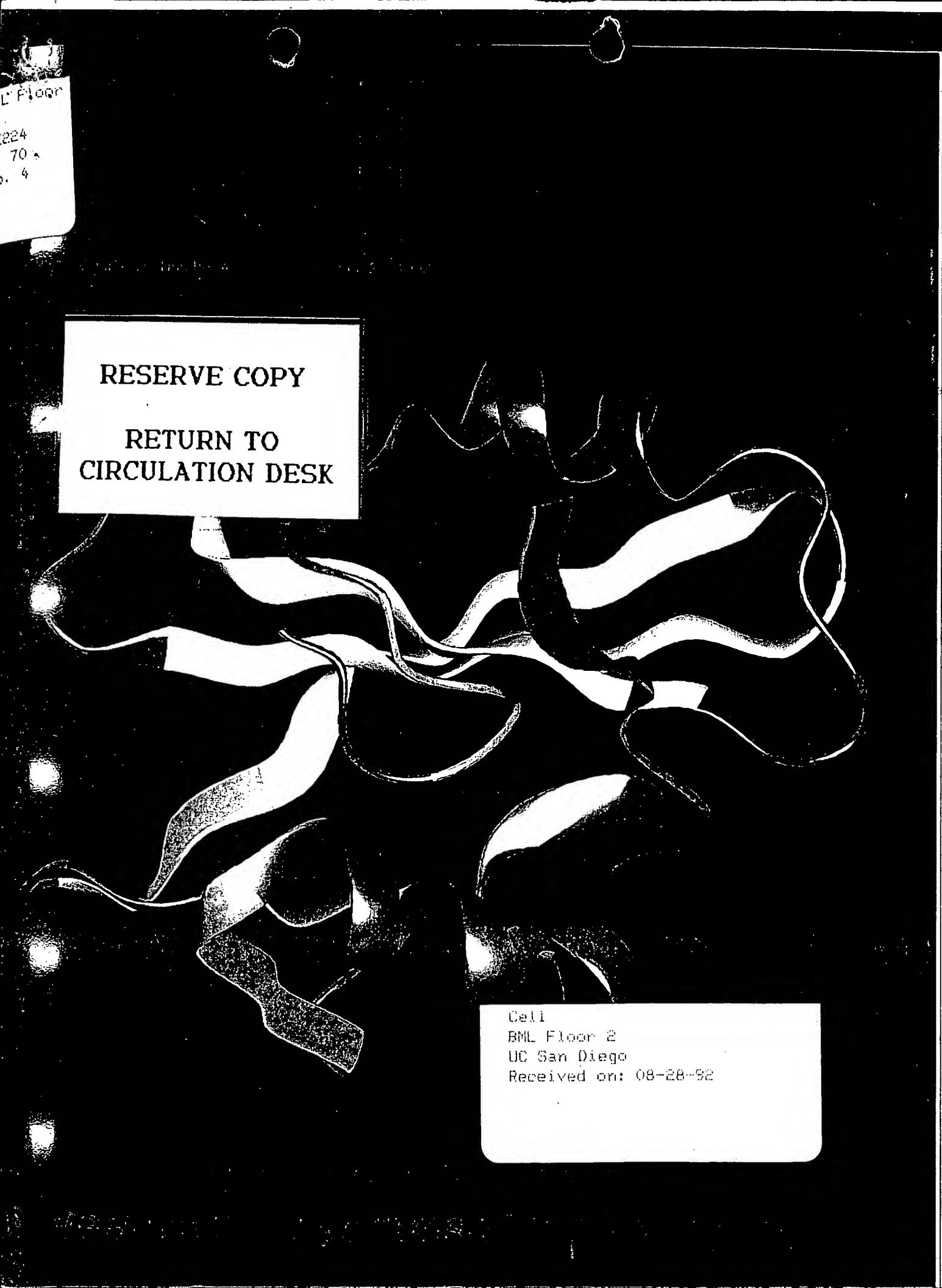
17. The method of claim 1, wherein said wild-type p53 gene is introduced to said tumor cell by direct injection.

18. The method of claim 1, wherein said wild-type p53 gene is introduced to said tumor cell by intra-arterial infusion.

19. The method of claim 1, wherein said wild-type p53 gene is introduced to said tumor cell by intracavitary infusion.

20. The method of claim 1, wherein said wild-type p53 gene is introduced to said tumor cell by intravenous infusion.

23. The method of claim 1, wherein said wild-type p53 gene is introduced to said tumor cell in aerosolized preparation.



Cell, Vol. 70, 523–526, August 21, 1992, Copyright © 1992 by Cell Press

p53 Function and Dysfunction

Minireview

discuss effect of "mutation" does not discuss the wt. gene replacing

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The word "cancer" is used to describe a group of heterogeneous pathologic states in which cells multiply abnormally and invade surrounding tissues. There are hundreds of different kinds of cancers, at least one originating from nearly every cell type in the mammalian organism. One long-standing hope has been that the same biochemical pathway for controlling growth is disrupted in many different kinds of cancers, despite their biologic heterogeneity; this would provide a common denominator for understanding, treating, and preventing these diseases. The pathway involving p53 fulfills this hope, as alterations of this tumor suppressor gene appear to be involved, directly or indirectly, in the majority of human malignancies. This has in turn stimulated an intense search for the biochemical functions of p53 and the effects of mutation on these properties.

Biochemical Activities of the Wild-Type p53 Protein

Two lines of p53 investigation have converged in the past year. First, it was noted that p53 contained an acidic domain near its N-terminus that was similar to those previously noted in well-characterized transcription factors (Fields and Jang, 1990; Raycroft et al., 1990). When this acidic domain was fused to the DNA-binding region of GAL4, the resulting chimeric protein could activate transcription from a *GAL4* operon. The activation domain has been recently mapped to the region lying between codons 20 and 42 (Unger et al., 1992; Miller et al., 1992) (Figure 1). Although many proteins contain such acidic regions, the strength of the activation and the nuclear localization of p53 suggest that p53 is involved in transcriptional control, either directly or through a complex with other proteins that bind to specific genes.

The second line of investigation illuminates this latter point. Through the testing of a large number of human genomic DNA clones, several have been identified that can bind to p53 in vitro (Kern et al., 1991; El-Deiry et al., 1992). Mapping of the p53-binding sites within these clones reveals that each contains two copies of the 10 bp motif 5′-PuPuPuC(A/T)(A/T)GPyPyPy-3′. One copy of the 10 bp motif is insufficient for binding, but binding is preserved when the two copies are separated by up to 13 bp of random sequence. The p53-binding sites have an obvious symmetry—four copies of the half-site 5′-(A/T)GPyPyPy-3′ are oriented in opposing directions. This suggests that p53 may bind to these sites as a tetrameric protein, which is consistent with biophysical studies indicating that p53 exists as a tetramer in solution (Stenger et al., 1992).

Two additional studies have confirmed that p53 can specifically bind to such sequences. From a large pool of random oligonucleotides, a small subset is selectable by virtue of binding to p53 (Funk et al., 1992). These synthetic oligomers share a 20 bp sequence very similar to the p53-binding sites described above. Anti-p53 antibodies and unidentified proteins from nuclear extracts appear to stabilize the complex of p53 with its binding sites (Funk et al., 1992; El-Deiry et al., 1992). The SV40 genome also contains a weak binding site for p53, which matches the 20 bp p53-binding site at 16 positions (Bargonetti et al., 1991).

If p53 binds DNA specifically and contains an acidic activation domain at its N-terminus, one would expect that p53 could activate the expression of genes adjacent to a p53-binding site. This expectation has been confirmed: cotransfection of a p53 expression vector with a plasmid containing a p53-binding site upstream of a reporter gene results in a high level of reporter activation in mammalian cells (Kern et al., 1992; Funk et al., 1992). This activation could have been an indirect one, perhaps in response to the numerous changes in gene expression and growth parameters induced by artificially high levels of wild-type p53. Indeed, p53 has been shown to affect the expression of several genes, few of which are likely to contain p53-binding sites (Ginsberg et al., 1991; Weintraub et al., 1991). Several additional experiments, however, strongly argue that p53 can directly activate transcription from p53-binding sites. First, the level of transcriptional activation precisely correlates with the strength of binding to p53-binding sites in vitro (Kern et al., 1992). Second, p53-binding site–mediated transactivation of reporter genes by p53 can be observed in yeast as well as in mammalian cells (Scharer and Iggo, 1992; Kern et al., 1992). If such effects are indirectly mediated through the induction of another gene product that actually binds to p53-binding sites in vivo, then the induction and function of this second gene product would have to be remarkably conserved in evolution, even though there is no known p53 homolog in

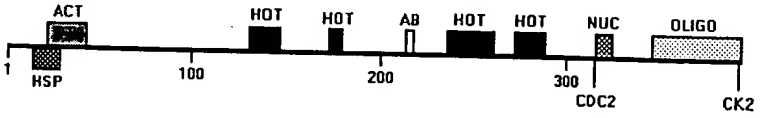

Figure 1. p53 Domain Map

HSP, a domain (amino acids 13–29) implicated in binding of heat shock proteins to mutant p53 (Lam and Calderwood, 1992); ACT, domain (amino acids 20–42) that, when fused to the DNA-binding domain of GAL4, activates transcription of genes downstream from GAL4-binding sites (Fields and Jang, 1990; Raycroft et al., 1990; Unger et al., 1992); HOT, "hotspots" (amino acids 129–146, 171–179, 234–260, 270–287) corresponding to evolutionarily conserved domains containing the most frequent sites of missense mutation (Hollstein et al., 1991); AB, region (amino acids 213–217) binding to mAb240 in some p53 mutants (Stephen and Lane, 1992); NUC, major nuclear localization signal (amino acids 316–325; Shaulsky et al., 1990); CDC2, serine-315 phosphorylated by $p34^{cdc2}$ kinase (Bischoff et al., 1990); CK2, serine-392 phosphorylated by casein kinase 2 (Fakharzadeh et al., 1991); OLIGO, domain (amino acids 344–393) required for p53 oligomerization (Milner and Medcalf, 1991). Human p53 contains 393 amino acids.

yeast. Third, transcriptional activation dependent on p53-binding sites can be demonstrated in an in vitro system using nuclear extracts and purified p53 (Farmer et al., 1992).

The Effect of p53 Mutations

The studies described above suggest that transcriptional activation through p53-binding sites is an important and biochemically assessable feature of the normal p53 protein. If this feature were central to the ability of p53 to suppress neoplastic growth, one might expect it to be disrupted by all p53 gene mutations. A large number of human p53 mutants have been described, the majority occurring as missense changes in one of the four "hotspots" shown in Figure 1. Representative mutants from each of these four regions have been tested for binding to p53-binding sites in vitro and for activation of p53-binding site reporter gene expression in vivo and in vitro. All mutants lose the ability to bind p53-binding sites and accordingly cannot activate the expression of adjacent reporter genes (El-Deiry et al., 1992; Kern et al., 1992; Scharer and Iggo, 1992; Farmer et al., 1992).

In addition to uniformly losing the ability to bind p53-binding sites, some mutants seem to change the global conformation of p53. For example, some missense mutants of p53 expose an epitope centered at codons 213–217, allowing it to react with monoclonal antibody 240 (Stephen and Lane, 1992). Similarly, some mutants of p53 allow binding to heat shock proteins (Sturzbecher et al., 1987), and others alter the acidic activation domain so that it cannot function when fused to the DNA-binding domain of GAL4 (Fields and Jang, 1990; Raycroft et al., 1990; Unger et al., 1992). Because these properties are found in only a fraction of p53 mutants, they are unlikely to be central to p53 function. However, the observations suggest that subtle mutations of p53 can affect the conformation of the entire protein, altering the structure of domains far removed from the sites of mutation (see Figure 1).

Such changes in conformation appear not only to affect p53 mutant molecules, but also can affect wild-type molecules complexed with the mutant proteins within the tetramer. Thus, cotranslation of mutant and wild-type proteins results in a change in conformation of the wild-type protein that mimics that of the mutant (Milner and Medcalf, 1991). Complexes of wild-type and mutant p53 protein cannot bind p53-binding sites in vitro or transcriptionally activate p53-binding site reporter genes in vitro or in vivo (Kern et al., 1992; Farmer et al., 1992). The binding to and consequent inactivation of wild-type p53 could explain the fact that some mutants of p53 can neoplastically transform cells, presumably by inhibiting endogenous wild-type p53 function in a dominant negative fashion. However, these dominant negative effects are not shared by all p53 mutants. p53 mutations that result in truncations will not exhibit dominant negative effects, because oligomerization is dependent on the presence of an intact C-terminus (Milner and Medcalf, 1991) (Figure 1). Moreover, some missense mutations exert more potent dominant negative effects than others and can be observed in vivo but not in vitro (Milner and Medcalf, 1991; Kern et al., 1992).

Genetic Alterations Affecting the p53 Pathway

In addition to intragenic mutations of p53, alterations of other genes apparently can lead to the same physiologic consequences. The first examples of this were provided by DNA tumor viral oncogenes, such as the large T antigen gene of SV40, the E1B gene of adenovirus, and the E6 gene of human papilloma virus. Each of these genes encodes proteins that bind to p53, and in the case of E6, this binding results in p53 degradation (Scheffner et al., 1990). Cells that express one of these viral oncoproteins and p53 cannot activate expression of p53-inducible reporter genes (Yew and Berk, 1992; J. A. Mietz and P. Howley, personal communication). The inhibition of p53-induced gene expression, and presumably of p53-mediated growth regulation, may be critical for virus replication and/or transformation.

The p53 pathway may also be disrupted by alteration of a cellular gene, *MDM2*. This gene was originally identified by virtue of its amplification in a spontaneously transformed mouse cell line (Fakharzadeh et al., 1991). The *MDM2* gene product has recently been shown to bind to p53 (Momand et al., 1992). As is the case with the viral oncoproteins, this binding appears to inhibit the ability of p53 to transactivate genes adjacent to p53-binding sites (Momand et al., 1992). This interference with p53 activity is not simply an experimental curiosity: the *MDM2* gene is amplified in a significant fraction of the most common human sarcomas, and the consequent overexpression of *MDM2* is likely to interfere with p53 activity (Oliner et al., 1992).

A Model for p53 Growth Control

The studies reviewed above suggest a model for p53 depicted in Figure 2. In this model, the wild-type p53 gene binds to p53-binding sites as a tetramer and stimulates the expression of downstream genes that negatively control growth and/or invasion. This expression can be lost in a variety of ways. In some tumors, a loss of one or both p53 alleles (through a large chromosomal defect or a localized deletion) reduces the concentration of p53 tetramers below that required to stimulate expression. In other tumors, a nonsense mutation results in the truncation of p53; the loss of the oligomerization domain at the C-terminus prevents the participation of the mutant p53 in tetramers. More commonly, one allele of p53 develops a missense mutation. This results in the reduction of tetramers composed totally of wild-type p53 monomers. Heterozygous mutant–wild-type tetramers do not function normally, and this dominant negative effect may be exacerbated by the increased stability (and therefore higher intracellular concentration) of the mutant protein compared with that of the wild-type protein.

A missense mutation of one p53 allele is often accompanied by a deletion of the other allele (usually through mitotic recombination), resulting in the absence of any wild-type p53 tetramers. This occurs in many tumors, including those of the colon, brain, lung, liver, and bladder (Hollstein et al., 1991). In cervical cancers, the expression of E6 results in the functional inactivation of p53 through binding and degradation. In soft-tissue sarcomas, the amplifica-

MECH.	CELLS	GENE	ALTERATION
--	NORMAL	----	NONE
1	OCCASIONAL TUMORS OF SEVERAL TYPES	p53	DELETION OF ONE (OR BOTH) ALLELES
2	FAIRLY COMMON IN LUNG, ESOPHAGUS, AND OTHER CANCERS	p53	NONSENSE OR SPLICE SITE MUTATION RESULTING IN TRUNCATION
3	COMMON IN COLON, BRAIN, LUNG, BREAST SKIN, BLADDER AND OTHER CANCERS	p53	MISSENSE MUTATION
4	COMMON IN CERVICAL CANCERS	E6	HPV INFECTION
5	COMMON IN SARCOMAS	MDM2	AMPLIFICATION

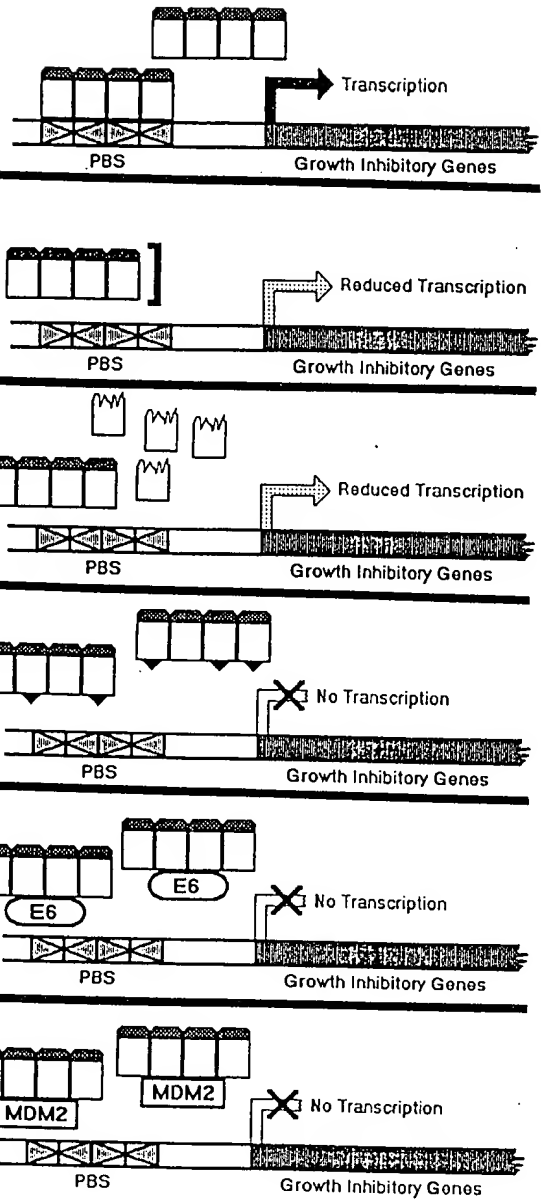

Figure 2. p53 Inactivation Mechanisms

p53 is postulated to bind as a tetramer to a p53-binding site (PBS) and activate the expression of adjacent genes that inhibit growth and/or invasion. Deletion of one or both p53 alleles reduces the expression of tetramers, resulting in decreased expression of these genes (mechanism 1). Mutations that truncate the protein do not allow oligomerization, thus resulting in a similar reduction of p53 tetramers (mechanism 2). Missense mutations resulting in dominant negative effects result in an even greater reduction of functionally active tetramers (mechanism 3). By binding to p53, the expression of E6 (mechanism 4) and increased expression of MDM2 (mechanism 5) result in functional inactivation of p53. It is not known whether E6–p53 and MDM2–p53 complexes inhibit binding to p53-binding sites, or whether they allow binding to p53-binding sites but inhibit transcriptional activation. E6 may also degrade p53 through ubiquitin-mediated proteolysis.

tion of *MDM2* and the binding of its product to p53 creates a similar loss of functional p53. In both cervical carcinomas with E6 expression and sarcomas with *MDM2* amplification, p53 mutations appear to be rare, whereas such mutations are common in other cervical cancers and sarcomas (Crook et al., 1992; Oliner et al., 1992). This is consistent with the expectation that only one mechanism for inactivating p53 in an individual tumor cell is required.

What Is Regulated by p53?

Although p53 can block the progression of the cell cycle when artificially expressed at high levels, it appears to play little role in normal cell cycle control. Thus, in mice

containing homozygous deletions and humans harboring germline mutations of p53, development is normal (e.g., Donehower et al., 1992; Malkin et al., 1990), and p53 protein is expressed at very low levels in most cell types. Emerging evidence, however, suggests that p53 may play an important growth-controlling role in stressed cells. In response to X-ray- or drug-induced damage, normal cells increase p53 expression and are arrested in the cell cycle until the damage is repaired. In contrast, cells with mutant p53 genes are only partially blocked, continue to divide, and then die (Kastan et al., 1991).

Developing tumor cells in situ may progress through a phase when they are stressed, perhaps as a result of anoxia or aneuploidy. As a result, wild-type p53 expression might be induced and limit growth, perhaps by stimulating p53-binding site–specific patterns of gene expression that inhibit cell cycle progression. Selection for mutant p53 genes at this juncture would allow further tumor expansion. This would explain why p53 mutations generally occur only late in tumor progression (Baker et al., 1990), when stress affords a selective advantage for cells containing p53 mutations; prior to this point, p53 is not expressed at significant levels and is not rate limiting for growth. It might also explain why tumor cells are often more sensitive to DNA-damaging agents such as those used in radiation and chemotherapy; this sensitivity may be a beneficial side effect of the loss of p53 function, which would otherwise limit cell death. p53 mutations may therefore constitute one of the few oncogenic alterations that increase rather than decrease the sensitivity of cells to antitumor agents.

Prospects for the Future

Among the most immediate issues is the identification of the genes adjacent to p53-binding sites that are regulated by p53 and that presumably control cellular growth. Although the data obtained so far have indicated that wild-type p53 can only directly affect gene expression through transcriptional activation, it is possible that p53 can also inactivate growth-promoting genes, just as other transcription factors can either activate or repress genes, depending on the sequence context of their binding sites. p53 is unlikely to interact with RNA polymerase directly; it will be important to discover the proteins that bridge p53 and this enzyme. In addition to MDM2, other proteins that negatively (or positively) affect p53 transcriptional control must exist, and it will be of interest to identify them. Alterations of the genes encoding these proteins could lead to a breakdown of the p53 pathway in the same way as *MDM2* in sarcomas. Finally, the three-dimensional structure of the p53 tetramer is likely to provide much important information. Particularly intriguing will be the elucidation of why the conformation of p53 and its functional properties are so easily disrupted by subtle mutations.

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